
LABORATORY ACTIVITIES SUMMARY SUPPLEMENTAL INFORMATION

The following is a description of the laboratory activities supplemental to the information provided in the report, including sample preparation, filtration, extraction, spike recoveries, and reporting limits. NEIC laboratory activities and additional information on these analyses are documented in the project file.

SAMPLE PREPARATION

For each sample, mass-labeled per- and poly-fluoroalkyl substances (PFAS) analytes were added to a 750-milliliter (mL) subsample. The addition of mass-labeled PFAS to these subsamples helps to compensate for extraction and response variability during sample extraction and analysis. Ideally, a target analyte is paired with its mass-labeled analog (i.e., perfluorobutyric acid (PFBA) paired with mass-labeled PFBA). For some of the target analytes, no mass-labeled analog was available, so these analytes were paired with another mass-labeled analyte of the same compound class. An example of a “mismatch” pairing was perfluoropentanesulfonic acid (PFPeS) paired with mass-labeled perfluorobutanesulfonic acid (PFBS).

SAMPLE FILTRATION

Each subsample was passed through a glass fiber filter in order to remove any sediment that may be present. Sediment removal was critical, as the subsample would be passed through a solid phase extraction (SPE) cartridge to extract the PFAS analytes; any sediment material present would disrupt and potentially stop the liquid flow through the cartridge.

The spent filter from each of the three trip blank and field spike samples, the rinse and field blanks (VP1364-09, VP1364-11) and site samples VP1364-07, VP1364-12, VP1364-16, and VP1364-18, indicated little or no sediment present in these sample, while a noticeable amount of sediment was removed from site samples VP1364-08, VP1364-10, VP1364-13, VP1364-14, and VP1364-17.

As for VP1364-15 (Outfall A01 (Pond #3)), the 750-mL subsample was initially passed through two glass fiber filters to remove any sediment. When this filtered subsample was being passed through the SPE cartridge, the liquid flow stopped after a few minutes because the cartridge had become plugged with sediment material. The remaining subsample was then passed through three additional filters, and the resulting filtered subsample was then passed through a second SPE cartridge without incident.

SAMPLE EXTRACTION, AND INJECTION SOLUTIONS

For each sample, approximately 500 mL of the filtered subsample was pass through an SPE cartridge that would remove PFAS and other materials from the water. Afterward, the cartridge was then washed with a buffered aqueous solution, and a vacuum was used to remove residual aqueous solution from the cartridge. Methanol solutions were passed through the cartridge to remove the retained materials including PFAS analytes, and these extracts were further concentrated using nitrogen gas and a warm water bath.

The sample extraction method referred to in **Table 4** of the report was modified for these analyses as shown below.

- Calibration standards were prepared prior to solution injection into the system. According to National Exposure Research Laboratory (NERL) methods, the calibration standards were prepared and extracted in the same manner as the field samples, and the diluted extracts were then injected into the mass spectrometer systems.
- A five-station manifold system that used nitrogen gas to push the liquid sample through the solid phase sorbent beds was used during the sample extraction process, instead of a dual-piston pump assembly.
- Filtered subsample aliquots and extract aliquots were diluted 1:1 with 2mM NH₄OAc solutions rather than 1:3.

LC/MS QQQ ANALYSIS

Target Analytes and Reporting Limits

For each analyzed solution, observed analyte concentrations were calculated for those PFAS that were identified, and a calculated reporting limit was determined for each target analyte, based on sample preparation and the most dilute calibration standard.

For each analysis set (SPE extracts, Diluted #1, Diluted #2), results from the three trip blanks, the two laboratory blanks, the equipment rinse and field blank samples were evaluated in determining a reporting limit (R.L.) value for each analyte. Because PFAS are ubiquitous, some target analytes were present in quantifiable amounts, in one or more of the blank sample extracts. Based on these results, the following criteria was used in determining R.L. values for each analysis set:

1. If a quantifiable amount of a target analyte was present in one or more of the seven blank samples, the largest concentration value was the R.L. value for that analyte.

2. If no quantifiable amount of a target analyte was present in any of the seven blank samples, the blank sample(s) having the largest calculated reporting limit was used as the R.L. value for that analyte.

Field Spike Sample Recoveries

As previously mentioned, three field spike samples prepared and analyzed with the other site samples, and these samples contained the PFAS target analyte shown in **Table 7** of the report, except for PFHxDA and PFODA. **Table 1** shows the spiked analyte concentration in nanograms per liter (ng/L) and percent recovery results from the SPE extract analysis set, and **Table 2** shows the recovery results from the Diluted #1 analysis set.

Table 1. TARGET ANALYTES AND RECOVERIES FROM SAMPLES VP1364-04, VP1364-05, AND VP1364-06: SPE EXTRACTS ANALYSIS SET							
Compound Class	Analyte	Analyte Concentration (ng/L)					
		VP1364-04		VP1364-05		VP1364-06	
		Target	Recovery (%)	Target	Recovery (%)	Target	Recovery (%)
Carboxylic acids	PFBA	25.0	113	50.0	108	100	109
	PFPeA	25.0	104	50.0	103	100	104
	PFHxA	25.0	107	50.0	107	100	107
	PFHpA	25.0	96.6	50.0	95.3	100	95.8
	PFOA	25.0	118	50.0	104	100	106
	PFNA	25.0	117	50.0	102	100	102
	PFDA	25.0	106	50.0	109	100	106
	PFUnA	25.0	65.5	50.0	72.3	100	61.8
	PFDoA	25.0	73.0	50.0	68.2	100	65.2
	PFTra*	25.0	Not observed	50.0	Not observed	100	166
	PFTeA	25.0	Not observed	50.0	Not observed	100	Not observed
Ether carboxylic acids	GenX	25.0	114	50.0	108	100	101
	PFECA-A*	25.7	115	51.4	99.4	103	93.0
	PFECA-B*	25.0	98.8	50.1	85.6	100	81.6
	PFECA-G*	26.1	62.4	52.3	51.5	105	46.8
Sulfonamides	FOSA	25.0	120	50.0	126	100	121
	N-MeFOSA	25.0	Not observed	50.0	Not observed	100	75.5
	N-EtFOSA	25.0	Not observed	50.0	Not observed	100	Not observed
Sulfonamidoacetic acids	N-MeFOSAA	25.0	56.7	50.0	67.0	100	64.4
	N-EtFOSAA	25.0	66.0	50.0	59.7	100	54.9
Sulfonamidoethanols	N-MeFOSE	25.0	86.7	50.0	74.5	100	81.8
	N-EtFOSE	25.0	63.5	50.0	50.2	100	65.4
Sulfonic acids	PFBS	22.1	105	44.3	104	88.5	105
	PFPeS*	23.5	61.7	47.0	63.8	94.0	65.0

Table 1. TARGET ANALYTES AND RECOVERIES FROM SAMPLES VP1364-04, VP1364-05, AND VP1364-06: SPE EXTRACTS ANALYSIS SET							
Compound Class	Analyte	Analyte Concentration (ng/L)					
		VP1364-04		VP1364-05		VP1364-06	
		Target	Recovery (%)	Target	Recovery (%)	Target	Recovery (%)
	PFHxS _{branched}	4.30	83.8	8.60	95.0	17.2	102
	PFHxS _{linear}	18.5	117	37.0	113	74.0	109
	PFHpS*	23.8	177	47.5	184	95.0	185
	PFOS _{branched}	4.89	691	9.78	126	19.6	196
	PFOS _{linear}	18.3	539	36.5	155	73.0	215
	PFNS*	24.0	36.7	48.0	41.5	96.0	43.6
	PFDS*	24.1	27.7	48.3	25.5	96.5	30.8
Telomer sulfonates	4:2 FTS	23.4	103	46.8	105	93.5	105
	6:2 FTS	23.8	108	47.5	109	95.0	109
	8:2 FTS	24.0	99.5	48.0	83.4	96.0	88.0
	10:2 FTS*	24.1	26.2	48.2	31.0	96.4	31.3

*- Mismatch pairing: mass-labeled analyte was not an analog of the target analyte

Table 2. TARGET ANALYTES AND RECOVERIES FROM SAMPLES VP1364-04, VP1364-05, AND VP1364-06: DILUTED #1 ANALYSIS SET							
Compound Class	Analyte	Analyte Concentration (ng/L)					
		VP1364-04		VP1364-05		VP1364-06	
		Target	Recovery (%)	Target	Recovery (%)	Target	Recovery (%)
Carboxylic acids	PFBA	25.0	178	50.0	86.1	100	103
	PFPeA	25.0	< R.L.	50.0	89.2	100	95.5
	PFHxA	25.0	115	50.0	116	100	120
	PFHpA	25.0	Not observed	50.0	111	100	98.6
	PFOA	25.0	101	50.0	87.2	100	93.2
	PFNA	25.0	138	50.0	100	100	91.1
	PFDA	25.0	Not observed	50.0	Not observed	100	Not observed
	PFUnA	25.0	Not observed	50.0	Not observed	100	Not observed
	PFDoA	25.0	Not observed	50.0	Not observed	100	Not observed
	PFTra*	25.0	Not observed	50.0	Not observed	100	Not observed
Ether carboxylic acids	GenX	25.0	Not observed	50.0	136	100	114
	PFECA-A*	25.7	126	51.4	106	103	107
	PFECA-B*	25.0	Not observed	50.1	116	100	84.3
	PFECA-G*	26.1	< R.L.	52.3	67.7	105	100
Sulfonamides	FOSA	25.0	200	50.0	< R.L.	100	119
	N-MeFOSA	25.0	Not observed	50.0	Not observed	100	Not observed

Table 2. TARGET ANALYTES AND RECOVERIES FROM SAMPLES VP1364-04, VP1364-05, AND VP1364-06: DILUTED #1 ANALYSIS SET							
Compound Class	Analyte	Analyte Concentration (ng/L)					
		VP1364-04		VP1364-05		VP1364-06	
		Target	Recovery (%)	Target	Recovery (%)	Target	Recovery (%)
	N-EtFOSA	25.0	Not observed	50.0	Not observed	100	Not observed
Sulfonamidoacetic acids	N-MeFOSAA	25.0	Not observed	50.0	Not observed	100	Not observed
	N-EtFOSAA	25.0	Not observed	50.0	Not observed	100	Not observed
Sulfonamidoethanols	N-MeFOSE	25.0	Not observed	50.0	Not observed	100	Not observed
	N-EtFOSE	25.0	Not observed	50.0	Not observed	100	Not observed
Sulfonic acids	PFBS	22.1	94.2	44.3	104	88.5	109
	PFPeS*	23.5	142	47.0	118	94.0	112
	PFHxS _{branched}	4.30	Not observed	8.60	< R.L.	17.2	< R.L.
	PFHxS _{linear}	18.5	141	37.0	97.2	74.0	84.9
	PFHpS*	23.8	135	47.5	89.7	95.0	55.3
	PFOS _{branched}	4.89	Not observed	9.78	Not observed	19.6	326
	PFOS _{linear}	18.3	Not observed	36.5	Not observed	73.0	188
	PFNS*	24.0	Not observed	48.0	Not observed	96.0	Not observed
	PFDS*	24.1	Not observed	48.3	Not observed	96.5	Not observed
Telomer sulfonates	4:2 FTS	23.4	Not observed	46.8	93.0	93.5	Not observed
	6:2 FTS	23.8	Not observed	47.5	Not observed	95.0	Not observed
	8:2 FTS	24.0	Not observed	48.0	Not observed	96.0	Not observed
	10:2 FTS*	24.1	Not observed	48.2	Not observed	96.4	Not observed

*- Mismatch pairing: mass-labeled analyte was not an analog of the target analyte

The recovery values presented in **Table 1** show acceptable results for many of the target analytes with some notable exceptions:

1. Except for PFECA-A and PFECA-B, recovery results from mismatched target/mass-labeled pairings were higher or lower than expected. These results may be due to extraction efficiency differences between the target and mass-labeled analytes.
2. Little or no analyte retention on the SPE cartridge during sample elution through the cartridge may be the reason for “Not observed” results for PFTrA, PFTeA, N-MeFOSA, and N-EtFOSA.
3. The high recovery results for the PFOS isomers may be a combination of extraction efficiency along with lower-than-anticipated mass-labeled PFOS area.

The recovery results in **Table 2** show several “Not observed” results, which were likely due to:

1. The analyte concentrations in these diluted subsamples are half of the sample concentrations (25 ng/L, 50 ng/L, and 100 ng/L), which are at the low end of the

calibration range, and matrix effects may partially or completely suppressed the responses.

2. The acid content in the filtered aliquots was 0.17% nitric acid, which may have affected the anion formation of these analytes before they entered the mass spectrometer.
3. These PFAS analytes must be in a negative charged state in order to be detected by the mass spectrometer.

LC/MS QTOF ANALYSIS

SPE sample extracts were initially analyzed in MS scan mode to determine if any of the analytes of interest were present based on mass to charge (m/z) ratio of the anion. Standards from the 3M Company were received, and the method modified to include these compounds. The sample extracts were then analyzed in targeted MS/MS mode using the most abundant ion at the appropriate retention time.

Deviations to the NERL Method for the QTOF Analysis:

- Due to response contributions from the labeled compound to native analyte response from the MS/MS transitions that were observed during method development, identification of GenX and m3GenX were done using MS data for the dimer of GenX (658.9439 [2M-H]⁻) and the dimer of m3GenX (664.9637 [2M-H]⁻).
- Source conditions were optimized:
 - Gas and sheath temperature: 125 and 175 degrees Celsius (°C)
 - Gas and sheath flow: 15 and 12 liters per minute (L/min)
 - Nebulizer: 20 pounds per square inch gauge (psig)

Data quality summaries and additional information for all laboratory measurements are maintained in the project file.